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Imaginal discs: Renaissance of a model for regenerative biology

*Cora Bergantiños, Xavier Vilana, Montserrat Corominas, and Florenci Serras**

Facultat de Biologia, Departament de Genètica, Institut de Biomedicina de la Universitat de Barcelona (IBUB), Universitat de Barcelona, Barcelona, Spain

Many animals display a capacity to regenerate tissues or even a complete body. One of the main goals of regenerative biology is to identify the genes and genetic networks necessary for this process. *Drosophila* offers an ideal model system for such studies. The wide range of genetic and genomic approaches available for use in flies has helped in initiating the deciphering of the mechanisms underlying regeneration, and the results may be applicable to other organisms, including mammals. Moreover, most models of regeneration require experimental manipulation, whereas in *Drosophila* discrete domains can be ablated by genetically induced methods. Here, we present a summary of current research into imaginal disc regeneration and discuss the power of this tissue as a tool for understanding the genetics of regeneration.

Keywords: development; *Drosophila*; imaginal discs; regeneration

Introduction

Regeneration is the ability of an organism to rebuild a part of its body that has been damaged or completely amputated, and covers a wide range of phenomena, from tissues that simply heal a wound after injury to organs that can completely regenerate. The phenomenon of regeneration has fascinated scientists throughout history, but regeneration research only joined the nascent field of modern experimental biology when naturalists adopted systematic approaches. The most intriguing aspect of tissue and organ regeneration is the capacity to reconstruct whole structures such as limbs in insects and amphibians, fins in fishes and heads in flatworms and hydra.[1,2] This raises the question of how pattern formation is achieved during regeneration and whether regeneration bypasses or reuses developmental circuits. Some of the most relevant processes of pattern formation and morphogenesis have been uncovered in *Drosophila melanogaster* using advances in molecular biology, genetic engineering, and genetic analysis. Moreover, *Drosophila* makes an ideal organism in which to study the genetics of regeneration, as it is relatively complex compared to other model systems and uses many developmental mechanisms similar to those utilized in vertebrate development.

***Correspondence to:** F. Serras, Facultat de Biologia, Departament de Genètica, Universitat de Barcelona, Av. Diagonal 645, 08028 Barcelona, Spain.
E-mail: fserras@ub.edu

Drosophila imaginal discs provide a particularly well-characterized experimental system in which to study regeneration.[3–5] These are larval epithelial sacs that contribute to adult cuticular structures.[6] Imaginal disc precursors are small groups of embryonic ectodermal cells.[7] These primordial cells proliferate extensively and invaginate to form the imaginal discs during larval development. By the end of larval stages, imaginal disc cells are committed to specific fates. During metamorphosis, while larval cells enter apoptosis, the imaginal discs undergo major morphogenetic changes to form the adult legs, wings, eyes, antennae, head capsule, halteres, and genital organs.

Haynie and Bryant[8] showed that, although irradiation of larvae results in the death of more than 50% of cells, including imaginal disc cells, a normal fly develops. Moreover, as occurs in amphibian limbs,[9] a regeneration blastema forms after cutting a piece of disc,[3] and when isolated this can regenerate the lost structure.[10] These and many other observations show that imaginal discs have the capacity to regenerate after injury or cell death.

Here, we review current research into imaginal disc regeneration, especially in leg and wing discs, and discuss recent advances that present *Drosophila* imaginal discs as an emerging model in which to study the cellular, genetic and molecular basis of regeneration.

The origin of experimental regenerative biology in imaginal discs

There are many examples of insects capable of regenerating autotomized or amputated legs.[11,12] However, some of the basic principles and models for insect regeneration come from experimental observations in *Drosophila* imaginal discs. Imaginal disc fragments can be cultured for several days in the abdomen of adult females, where they proliferate but do not differentiate.[13,14] In contrast, discs transplanted into third instar larvae will metamorphose with the larvae and enter

differentiation. Thus, by combining these types of transplant, we can study regeneration: fragmented discs recovered from culture in adult hosts can be transplanted into larvae and the adult cuticle structures scored after metamorphosis (Fig. 1).

Detailed analysis of the differentiated adult cuticle reveals that cultured disc fragments not only differentiate according to their determined fate, but are also capable of giving rise to new tissue. Disc cells are fate specified during development, before fragmentation and transplantation,[3,5,15] and they have become a prototype of rigidly determined and invariant development. Fate maps of the leg[16] and the wing disc[17,18] indicate that they are organized like collapsed telescopes, with distal structures of the adult cuticle (claws and distal tip of the wing, respectively) derived from the center of the imaginal disc and proximal structures (coxa and wing hinge, respectively) from the periphery. As in regenerating flatworms, amphibian limbs, tadpole tails, and zebrafish fins (reviewed in ref.[9,19]), fragmented imaginal discs have been found to regenerate following a sequence of events that include wound healing, localized proliferation and repatterning of the lost tissue. Localized proliferation in regenerating discs was soon associated with the formation of blastemas, which in many regeneration models have been defined as a mass of undifferentiated cells from which an organ or body part grows.[20] Moreover, discs cultured for a long period of time, achieved by subjecting them to several rounds of transplantation into host abdomens, can switch to a different developmental program in a process known as transdetermination.[21] All these observations indicate that cell fates, which are pre-determined and can be mapped, are not restricted until differentiation is initiated.

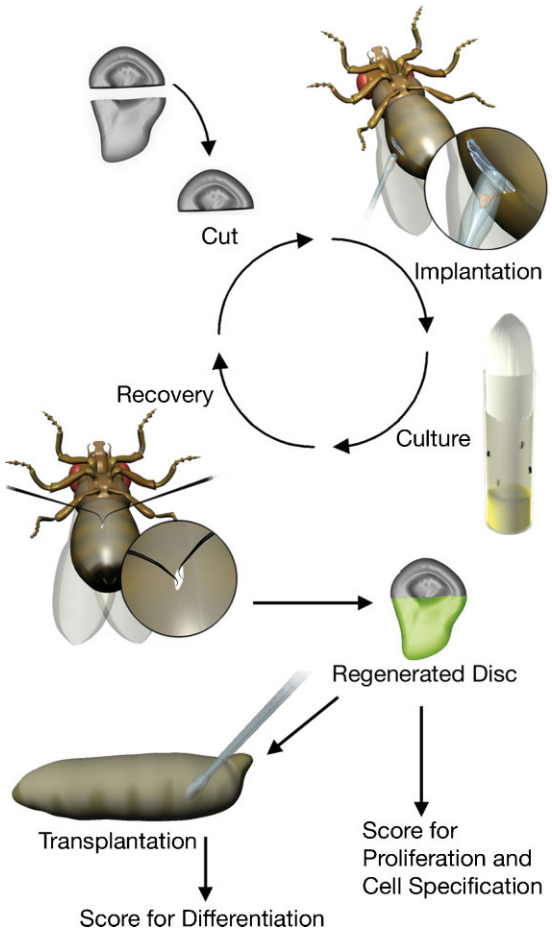

Figure 1. The classical method for studying regeneration in *Drosophila* imaginal discs. Discs are microsurgically fragmented. Fragments are implanted into the abdomen of an anesthetized host fly using a micropipette. Flies carrying discs are cultured in vials for several days. Regenerated discs can be recovered from hosts by microsurgery using tungsten needles. Regenerated discs can be either fragmented again and implanted for further culture, or used to score markers of cell specification and proliferation. To score for differentiation, they need to be transplanted into larvae. After metamorphosis, the discs differentiate into adult structures that can be identified in the cuticle.

Disc fragments regenerate or duplicate

Imaginal discs cut into fragments, cultured in adult hosts to allow proliferation, and transplanted into larval hosts to check for differentiation, display either regeneration of the missing structures or duplication of the existing ones.[4,5] For example, reciprocal fragments of bisected wing discs regenerate or duplicate accordingly to the topology of the fragment and position of the cut (Fig. 2A, B).[18] Thus, bisection along the disc periphery resulted in duplication of the small fragment and regeneration of the rest of the disc. This means that the decision to activate the regeneration or duplication program depends on the cellular context of the fragment. However, duplication is not as stable as regeneration. Many duplicating fragments are able to regenerate after duplication when the culture period is prolonged.[22,23] Interestingly, regenerated elements are produced during growth rather than during a change in cell specification of the old duplicated tissue.[24]

Regeneration can be driven by intercalation of lost tissue

When amputation results in the confrontation of two regions with different positional values, intercalary regeneration can replace the missing tissue. This accounts for leg regeneration in many organisms, including cockroaches and amphibians.[11,25–30] Imaginal discs also regenerate by intercalation (Fig. 2C): when two opposite peripheral pieces (which would duplicate when cultured separately) are cultured together, they reconstruct the missing central tissue.[31,32]

Based on intercalation of positional values, the polar coordinate model was proposed,[25] in which two coordinates

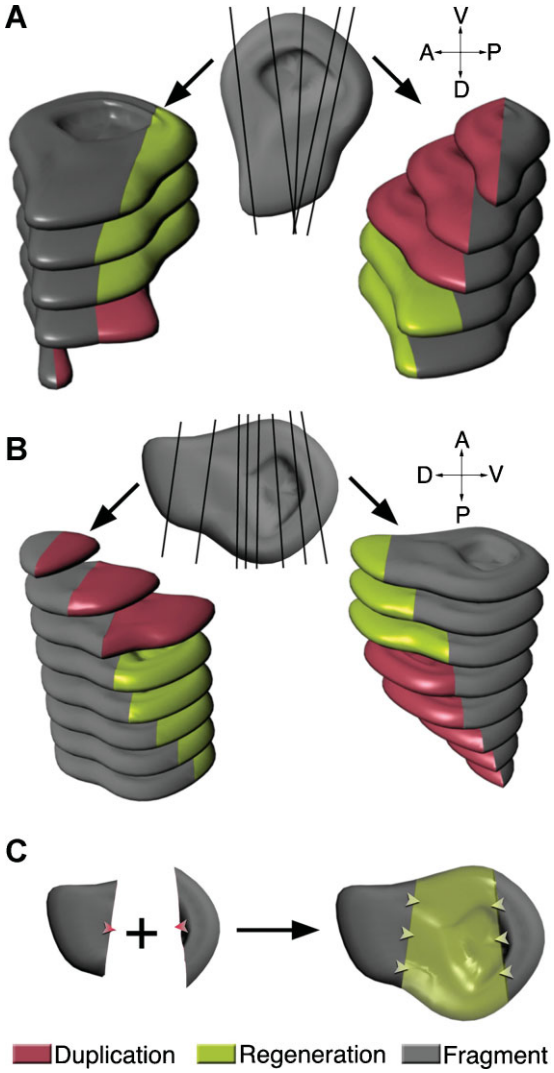

Figure 2. Interpretation of regeneration and duplication in wing discs. Model of discs bisected at different points along the dorsoventral (D-V) axis (**A**) or anteroposterior (A-P) axis (**B**).[18] Each cut (black lines) results in two halves (left and right stacks): one will regenerate (green) and the other duplicate (red). Note that in all stacks there is a transition from regeneration to duplication. Overlaying of the stacks reveals the limit of regenerative capacity. **C**: Model to illustrate regeneration by intercalation. When two pieces from the periphery of the disc that would normally regenerate are cultured together, regeneration is activated and intercalation of positional values occurs.[31]

define growth in either imaginal discs or limbs: (i) a circular component corresponding to the position around the outer boundary of the field (the disc limit, the leg cuticle or the skin), and (ii) a radial component equivalent to the position along the proximo-distal (Pr-Ds) axis, with the distal-most values placed in the center. When wound edges meet, the positional disparities would trigger either regeneration or duplication, according to the coordinates. However, questions were soon raised about the strength of this model because growth was found before healing was complete[10,33] and also because duplicated fragments are much smaller than regenerated ones, while the model predicts that these tissues should intercalate equally.[34]

Imaginal disc regeneration in the molecular era

One of the major contributions of imaginal disc research to the current body of knowledge has been the discovery of functionally distinct developmental units called compartments. These are stable domains delimited by boundaries that separate populations of cells with specific affinities and restrict cell mixing between adjacent compartments.[35–38] The boundaries separate cells in compartments along the two main body axes: anteroposterior (A-P) and dorsoventral (D-V). Topologically, imaginal discs – as well as the buds of insect legs and amphibian limbs – are flat fields. The third dimension, that is the Pr-Ds axis, would be created relative to the two existing boundaries. The distal-most point of the appendage is established in the spot of the flat field at which the three (in the leg disc: A-V, A-D, and P) or four (in the wing disc: A-V, A-D, P-V, and P-D) territories meet. This distal spot is located centrally, and the periphery of the field differentiates proximal structures.

Compartment boundaries function in growth, organization, and patterning of the disc by serving as signaling centers. Meinhardt[39,40] proposed the boundary model based on the assumption that intersections of compartment boundaries act as organizers of disc regeneration (Fig. 3A). Thus, regeneration or duplication would be the result of new compartmental border confrontation. Meinhardt's vision for regenerating discs and limbs was that the experimentally induced confrontation of different compartment boundaries would result in the production of morphogens responsible for repatterning. According to this model, regeneration requires that some cells of all territories enclosed within the A-P and D-V boundaries be present in the disc when some of its tissue is removed. If this is the case, wound healing would bring together all these territories and generate a new distal tip, thus determining a new Pr-Ds axis. Therefore, all the necessary information to create a complete set of elements of the disc will be present and the missing tissue can be reconstructed by intercalation. In contrast, total ablation of a compartment would result in duplication instead of regeneration.

This model is also applicable to the regeneration of the legs of cockroaches and crickets.[41–43] When a leg is amputated and grafted onto a collateral stump, regeneration will result in supernumerary limbs.[11] This operation results in juxtaposition of cell identities. For example, the dorsal and ventral regions of the leg are in register and the anterior and posterior

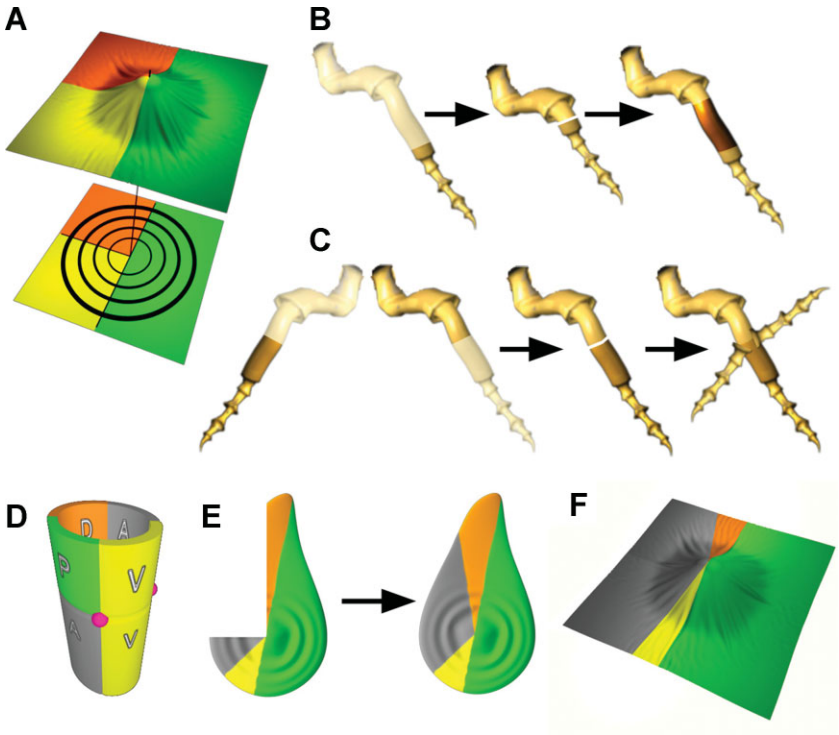

Figure 3. Axis formation in regenerating legs and leg discs. **A**: Diagram of the boundary model for a leg disc. A morphogen is produced at the intersection between cells of the anterior-dorsal (orange), anterior-ventral (yellow), and posterior (green) compartments. The local concentration of this morphogen results in a cone-shaped gradient that provides the positional information to form the proximo-distal axis (concentric rings). **B**,**C**: Bohn's[11,28] experiment on intercalation. A proximal stump normally regenerates distal structures. However, when a distal part of a leg is grafted onto a proximal stump (B), only the intermediate missing parts are intercalated (dark zone). When an amputated distal leg is grafted onto the contralateral amputated proximal leg (C), three axes develop distally. **D**: The interpretation of this graft is that some domains remain in register (ventral-ventral; V-V), whereas others do not (anterior-posterior; A-P). This creates new points of confrontation of between different boundaries (pink dots). Cells interpret this as the point at which a new axis should develop. **E**,**F**: According to Campbell and Tomlinson's[43] boundary model, the confrontation of Dpp (orange) and Wingless (yellow) originates the new axes. E: Leg disc fragment in which a quarter of the anterior compartment has been removed. The signaling sources of Dpp and Wg are intact and the disc regenerates normally and develops a normal leg disc. F: In that model, the leg Pr-Ds axis develops when Wg and Dpp expression domains are confronted. Green in (E) and (F) represents the Hh-expressing domain (posterior compartment). All these figures are based on Meinhardt[39] and Campbell and Tomlinson.[43]

domains will be misaligned (Fig. 3B, C). Similarly, supernumerary legs are formed when grafts are rotated 180°. The boundary model interprets these observations as new areas of confrontation created at the graft-host junction, resulting in supernumerary limbs (Fig. 3A–D).

Regulatory circuits may help us understand how forced confrontations of cells in response to surgical or genetic manipulations can be resolved into coherent and organized patterns. Leg disc development has been used to decipher the circuits and signaling in the Pr-Ds axis, and can serve as a model for regeneration (reviewed in ref.[44]). Patterning of both the D-V and Pr-Ds axes is directed by the secreted morphogens BMP/Dpp and Wnt/Wg.[45,46] In leg discs, *dpp* and *wg* are expressed in narrow dorsal and ventral wedges, respectively. Both wedges consist of anterior cells abutting the A-P compartmental boundary (Fig. 3E). The expression of these signals is under the control of secreted Hedgehog protein from the P compartment.[47] Campbell and Tomlinson[43] proposed that the boundary model could be explained by the combinatorial action of Wnt/Wg and BMP/Dpp signals, and that the intersection of their expression domains defines the distal tip of the appendage (Fig. 3F). Therefore, during regeneration, the reconstruction of the leg will depend on whether these signals are able to meet and create a new axis, or many axes if new signal confrontations are experimentally created (Fig. 3D, E).

It is possible that cells also retain a memory of their positional values or cellular identities. Dpp and Wg act in a concentration-dependent manner to organize the different fates along the Pr-Ds axis.[48] For example, *Distalless* (*Dll*) expression requires high levels of Dpp and Wg, which are present in the distal regions of the leg. However, in later stages of disc development, Wg and Dpp are no longer needed, although Dll expression is maintained. The localized

expression of Dll in the leg disc results from the synergistic interaction between two *cis*-regulatory elements.[49] One functions early to integrate Wg and Dpp signal and trigger Dll expression, while the other acts later, in the third instar larva, to maintain Dll expression independently of Wg and Dpp. Thus, it remains unclear whether *cis*-regulatory elements respond to morphogens or alternatively whether enhancers retain a memory of the organized pattern during regeneration.

Regeneration restores compartment boundaries

In addition to the requirement for signals from all compartments, reconstituting a disc also requires boundaries to be rapidly reconstructed so that different compartments can come into contact. In both leg and wing discs, clones of marked cells induced at the time of fragmentation are able to cross the A-P boundary only when induced before injury.[50,51] The frequency of clones able to cross the compartmental border is low, even when proliferation advantage is conferred using the Minute technique.[52] Without this proliferative advantage, cells crossing the boundaries will be rare.[53] Thus, blastema cells may lose their compartmental commitment transiently, but they become rapidly assigned to their compartment of origin. Moreover, proliferation is first associated with the wound, and later the damaged compartment also reactivates proliferation to adjust the size, suggesting that compartments act as regeneration units (Bergantiños *et al.*, submitted). This fits well with lineage restrictions found in amphibian limb regeneration, as axolotl blastema cells do not become pluripotent as previously proposed,[54–56] but retain a strong memory of their tissue origin.[57]

Wound healing recapitulates embryonic epithelial fusion events

After fragmentation, imaginal discs reestablish epithelial integrity by healing the wound. Discs consist of a columnar epithelium, from which adult structures differentiate, and a squamous epithelium, the peripodial membrane. When an injury is inflicted, both the columnar and squamous epithelia participate in the healing process.[58] The first response is contraction of the wound and reduction of the wound surface, which favors close apposition of the two layers and a transient establishment of heterotypic contacts.[59] After the initial stage of heterotypic healing, cells at both wound edges create homotypic contacts. Probably due to the lack of an appropriate surface, individual cell migration and tissue rearrangement are not involved in healing.[58,60] Instead, the epithelial sheets undergo cell-shape changes that stream both epithelia towards each other. Cytoskeletal rearrangements are also involved. An F-actin cable develops along the wound edges and filopodia are extended to close the wound.[61,62]

The mechanisms underlying this healing process strongly resemble the molecular machinery involved in epithelial fusion events that occur in normal development, such as the embryonic dorsal and thoracic closure reviewed in ref.[63] As in those morphogenetic events, the Jun N-terminal kinase (JNK) pathway plays an important role in early disc regeneration and wound healing. Thus, loss-of-function mutants for components of the JNK pathway compromise closure and regeneration of the cut discs.[61,62] The mechanism of wound closure seems to be a general feature of healing and is comparable to other post-injury events in *Drosophila* tissues such as embryos,[64] larvae,[65] and adult cuticle.[66]

Regeneration requires blastema formation

During imaginal disc regeneration, there is evidence for proliferation of cells that accumulate in the area near the wound and from which the regenerated structures arise. Experiments using fluorescently tagged cells have revealed that wound healing brings into close contact cells that were previously widely separated in the disc; these reach their original position by intercalation of newly formed tissue.[60] Regeneration involves local stimulation of proliferation,[67] and does not occur under conditions that prevent cell proliferation.[68] Direct cell counts of regenerating wing disc fragments also support this view,[69] as do volume measurements of cultured discs.[34] Cell proliferation after injury has been monitored through several methods that label cells both in the S and M phases of the cell cycle[33,53,60,67,70,71] (Fig. 4A). These studies revealed that the dividing cells are clustered in the region near the wound and that proliferation peaks 2–3 days after the cut. The high regenerative capabilities of disc blastemas are not only demonstrated by localized proliferation but also by the observation that isolated and cultured blastemas are able to regenerate and differentiate most of the lost structures in an orderly manner.[10]

A common perception is that blastemas are composed of undifferentiated cells that regenerate the missing portion. In flatworms, accumulation of stem cells near the blastema will trigger regeneration.[72–74] However, in other organisms, the blastema contains precursor cells with restricted potentials. In axolotl limbs, cell tracking experiments have established that the blastema is a heterogeneous pool of restricted progenitor cells from the outset of regeneration.[57] In zebrafish, heart regeneration is conducted by differentiation of progenitor cells pooled in the heart, rather than dedifferentiation.[75] In fly discs, blastema cells retain their compartment origin and contribute only to the reconstruction of the damaged compartment ([53]; Bergantiños *et al.*, submitted). Thus, regeneration plasticity is restricted in the blastema of flies and some vertebrates, and instead of reverting back to an early

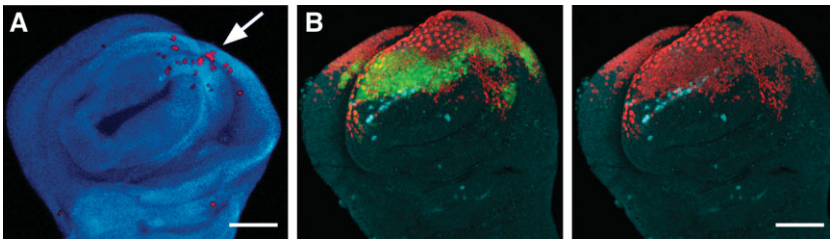

Figure 4. Blastema formation in wing discs. **A**: Wing disc in which a piece has been removed and cultured for 2 days. Immunostaining with anti-HP3 (red) reveals mitotic cells concentrated and localized in the region near the wound (arrow). **B**: JNK is activated near the wound, as revealed by *puckered* (*puc*) expression (green), which is a readout of the pathway. The flies used in this experiment contained the *puc-Gal4* transgene, which delivers the Gal4 transcription factor only in *puc*-expressing cells. The Gal4 protein binds to *UAS* sequences that drive *GFP* expression (green). Also, the same cells drive expression of the flipase recombinase *UAS-flp*. Here, the *flp* recombines the flipase-recombination targets (*FRT*) and, as a result, the ubiquitous promoter Act5c activates the *lac-Z* marker (red). Thus, cells derived from the *puc*-expressing domain are genetically labeled by *lac-Z* expression. The disc is 4 days old and the *puc* (JNK) domain is clearly visible and corresponds to the original borders of the wound. The red derivatives correspond to the reconstructed tissue. The same disc was stained with anti-Senseless (Sens) to mark the dorso-ventral boundary (blue). The right-hand panel shows the same image as in (B) with the green channel removed. Genotype of (B): *UAS-GFP/Act5c-FRT-stop-FRT-lacZ; puc-Gal4/UAS-FLP*. Scale bar: 50 μm.

pluripotent state, progenitor cells (amphibian limb and fish heart), or proliferating cells near the wound (fly discs) undergo reprogramming events to enter regeneration.

Sustained proliferation can switch to other genetic programs

Hadorn[21,76] tested whether the state of determination in imaginal discs was stable. He developed the long-term *in vivo* protocol that involved repeatedly culturing discs in female abdomens for long periods. He first cut a disc into two pieces: one piece, the ''test'' piece, was injected into a larva, where it differentiated allowing cell determination to be assessed. The other piece, the ''stem'' piece, was injected into the abdomen of an adult female. In this *in vivo* environment, disc cells proliferated with doubling times not significantly different from normal development. After 2 weeks, the grown fragment was isolated and cut again, and test pieces were injected into larvae and stem pieces into adults. He worked with the genital imaginal disc and found that in over 90% of cases, the test pieces differentiated genital structures. However, occasionally he observed leg bristles in addition to genital structures. This change in determination was called transdetermination. Transdetermination was observed with all imaginal discs and phenocopied gain and loss of homeotic selector genes. Initially, this was considered a rare event. However, later it was found that a cut through a specific disc region results in a high frequency of transdetermination after only a few cell divisions. This transdetermination-sensitive region was termed the weak point and was found in all imaginal discs.[77] Transdetermination from leg to wing is the most frequently studied example,[78] and it has been successfully reproduced by ectopic expression of *wg*.[79] Molecular dissection of this process points to the interaction of high levels of Dpp and Wg as the cause of fate switching in the dorsal anterior first leg fragment.[79–81] Cells of the weak point also transiently accumulate S phases, although they do not revert to a younger cell cycle profile, and vary their size when the developmental programs are switched.[82] Together, these observations indicate that cell determination in discs is labile and that some cells are capable of following alternative developmental programs.

Epigenetics of transdetermination and regeneration

Since a program of homeotic selector genes determines the identity of discs, transdetermination has been associated with inappropriate reprogramming of selector genes. The pattern of homeotic genes is set up in the early embryo by a cascade of transcriptional activators and repressors.[83] The maintenance of the identity of the discs throughout development is tightly associated to the maintenance of the disc-specific genetic programs. This is mainly accomplished by protein complexes, such as the trithorax and polycomb-group (PcG) genes, which act as transcriptional activators and repressors by interacting with and modifying the chromatin.[84] It seems plausible that the plasticity of discs can be related to changes in the activity of these complexes and therefore release of a different program. Two pieces of evidence point to the involvement of chromatin modification in transdetermination. First, the frequency of leg-to-wing transdetermination increases dramatically in heterozygous alleles of the silencer PcG genes.[85] Moreover, down-regulation of PcG function occurs in cells that actively proliferate and undergo transdetermination. This observation suggests that transdetermination leads to a permissive state for cell-fate switching driven by the loss of PcG silencing. Second, microarray analysis of transdetermining leg discs has unveiled several PcG and trithorax-group genes, indicating the importance of epigenetic modulation in transdetermination.[86]

In mammalian embryonic stem cells, PcG proteins silence expression of genes encoding developmental regulators and thereby maintain pluripotency, whereas selective relief of the PcG-silenced genes promotes differentiation.[87–89] It has been proposed that, as PcG-mediated repression is heritable, positional information might be regulated by the epigenetic modification inherited by blastema cells during amphibian limb regeneration.[90] Thus, it may well be that the restricted potential of regenerating tissues (*e.g.*, compartment identity in imaginal discs and positional identity in amphibian limb blastemas) requires epigenetic marks to maintain the memory of the missing parts and prevent cells from reverting to pluripotency. Thus, epigenetic regulation in transdetermination, where cell-fate switching can occur, could differ from that occurring in regeneration, where restricted fates must be preserved.

Current view of imaginal disc regeneration

Understanding the interplay between genetic and epigenetic constituents to shape gene regulatory networks during regeneration requires a versatile system that also permits genome-wide analysis of transcriptional regulation. A genetic screen, using a collection of enhancer-sensitive P-element insertions fused in-frame to a *lac-Z* reporter gene uncovered numerous loci that respond to disc regeneration.[91,92] In those studies, a temperature-sensitive cell-autonomous lethal allele of *suppressor of forked* was used, in which genetically induced cell death efficiently produces disc fragments that regenerate without the need for fragmentation or implantation. This screen identified *dpp* and the EGF repeat gene *crumbs*, which is necessary for epithelial integrity, as potential candidates. Thus, genetic induction of cell death seems to be a powerful alternative to microsurgery for studying regeneration. Moreover, activation of cell death can trigger compensatory proliferation in surrounding cells to recover the missing tissue. This principle has served to identify parallels between compensatory proliferation and regeneration in the imaginal discs.

Compensatory proliferation was discovered in discs about 30 years ago when it was found that irradiation at moderate doses can eliminate more than 50% of cells and yet the discs still recover their normal size.[8] The driving force that triggers compensatory proliferation has been the subject of several recent studies based on interference with the apoptotic response after irradiation or induction of pro-apoptotic genes. Essentially, cells that should die, but in which death is prevented by expressing the baculovirus protein p35,[93] remain in an "undead" state.[94–96] This facilitates analysis of the cell behavior in tissues that are in contact with cells that have entered but not completed the apoptotic program. Four principal observations of those undead cells are particularly noteworthy: (i) non-autonomous proliferation increases in compartments containing undead cells; (ii) mitogenic signals such as Dpp and Wg are liberated by apoptotic cells;[94–97] (iii) compartments do not lose their identity, although their borders are irregular;[98] and (iv) caspases can have a non-apoptotic role, acting as activators of compensatory proliferation.[94,97] In addition, the transcription factor dp53 is required for compensatory proliferation and its activity depends on the initiator caspase Dronc,[99,100] suggesting a role for p53 in maintaining the apoptotic identity of undead cells.

The mitogenic hypothesis of undead cells was initially based on the discovery of Dpp and Wg released by apoptotic cells, but their exact role in compensatory proliferation, if any, is still a mystery. Actually, in wing discs, Wg and Dpp downstream effectors are down-regulated instead of activated,[100] and compensatory proliferation occurs in the absence of Dpp and Wg signals produced by the apoptotic cells.[101]

Although the release of mitogens by apoptotic cells cannot be ruled out, the possibility that the normal process regulating compartment size during development could be reused to recover compartment size after damage is intriguing.[101] The A and P compartments are autonomous units of growth and size control.[102] Wing size and growth can be adjusted when cell size, cell numbers, or mitotic rates are genetically manipulated,[103–106] suggesting a homeostatic mechanism for wing disc size control.[107] Thus, compensatory proliferation is emerging as a cellular property that is activated after irradiation, apoptosis or microsurgical injury to trigger tissue repair and organ size control, as in normal growth, by adjusting the size within the damaged compartment.[101]

JNK activity in undead cells[95] has been found to be responsible for the activation of Dpp and Wg that drives hyperplastic growth of tissues near undead cells rather than for compensatory proliferation.[101] This JNK activity may be induced in cells held in an undead state for long periods. In contrast, it has recently been found that in cell death-induced regeneration, JNK is activated only in living cells near the wound and is required for healing and regeneration (Bergantiños *et al.*, submitted). In regeneration induced after microsurgery, JNK signaling activity, monitored by the expression of the JNK-responsive gene *puckered* (*puc*), is localized near the wound.[61,85] Importantly, cell-lineage experiments have revealed that derivatives of *puc*-expressing cells will reconstruct the lost tissue (Fig. 4B, C).[53] Thus, the activation of JNK occurs in blastema cells and can be considered as one of the first cell responses necessary to drive disc regeneration.

JNK has emerged as a regulator of the morphogenetic movement of epithelial sheets since, as mentioned, JNK signaling appears to be crucial in wound-activated tissue movements.[63] In *Drosophila,* a well-orchestrated JNK

signaling pathway controls formation of actin stress fibers and cell-shape changes, which are required for the sealing of embryonic epidermis, wound healing, and early regeneration. The JNK pathway is also involved in morphogenetic processes in mice, including closure of the eyelid, neural tube, and optic fissure.[108] Thus, it is likely that the epithelial response to injury triggers stress signals that respond by activating JNK, and that targets of this pathway will activate the leading network of regeneration regulators.

Conclusions and future prospects

The activation of JNK in regenerating discs indicates that very early changes and reprogramming occur near the damaged epithelia, at least in the early phase of regeneration. Compensatory proliferation may account for achieving the overall organ size of the damaged epithelium, but genetic and epigenetic cues will be crucial to control the repair. Expression profiles of regenerating discs have provided key clues to deciphering this genetic and epigenetic code.[86,109]

Experiments using regional promoters to activate Gal4/Gal80-inducible transgenes and induce cell death in particular domains of the disc will be of extraordinary value in analyzing early responses without the need for microsurgery and transplantation (Fig. 5). The application of this principle has recently allowed the identification of Wg and Myc as key players in disc regeneration.[110] Moreover, when the wing pouch is ablated, regenerating cells express Wg in a pattern that resembles a younger stage, and they progressively reconstitute all elements of the wing, including veins and interveins. This strongly suggests a reprogramming of the wing cells during regeneration. It remains to be determined whether all elements of the regenerated tissues come from existing precursors (*e.g.*, in wing discs, veins regenerate veins) or whether blastema cells can share restricted potentials (*e.g.*, a regenerating wing cell can choose between vein or intervein fate). There is no evidence of stem cells or precursor cells in regenerating imaginal discs. Rather, compartment boundaries are preserved or rapidly reestablished, which suggests that reprogramming of blastema or JNK-expressing cells occurs within each regenerating compartment. The cell response within the compartment after injury could be spread by short-range cell-to-cell contacts within the blastema, rather than by signaling gradients or circles. It is possible that damaged epithelia will reconstitute the missing part through a system of local interactions from boundaries or from other subsets of regions (*e.g.*, vein-intervein zones for wing disc).[111]

For regeneration studies, the cellular and molecular contexts of the imaginal discs are different from those of a regenerating salamander leg, hydra, planarian, or zebrafish heart. The main contribution of imaginal discs to our knowledge of regeneration will be in understanding the genetic basis of the early reactivation of proliferation and also the genetic and epigenetic circuits that lead to fate

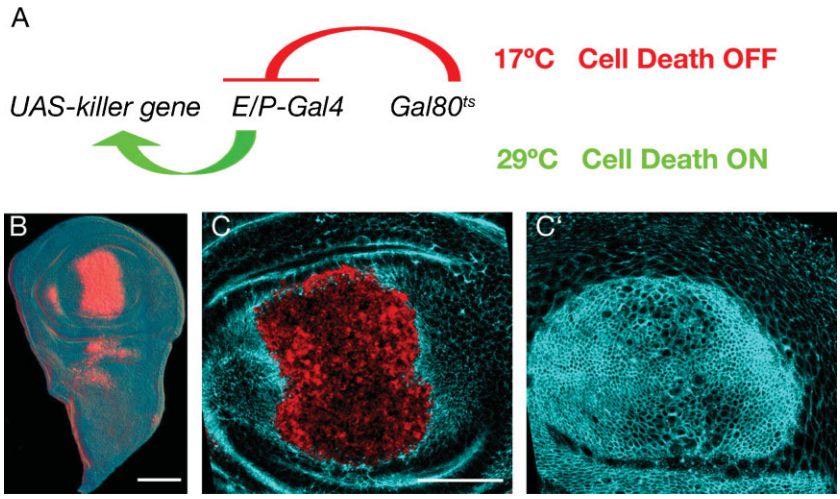

Figure 5. New approaches to studying regeneration after genetic elimination of specific domains in *Drosophila* imaginal discs. **A**: Expression of the Gal4 transcription factor is under the control of a tissue-specific enhancer. In that zone, Gal4 binds to UAS sites and activates a pro-apoptotic gene or a toxin (*UAS-killer gene*), and therefore promotes cell death. To avoid continuous death, a temperature-inducible form of Gal80, which acts as an inhibitor of Gal4, is used.[112] Thus, larvae cultured at 17°C grow normally, but when transferred to 29°C cell death ablates that particular region. Regeneration can be studied as the live surrounding tissue begins to reconstruct the missing part. **B**: Activation of the apoptosis promoter gene *reaper* (*rpr*) in the *spalt* domain of the wing disc results in extensive cell death (cleaved caspase-3 in red). **C**,**C'**: Confocal images of a wing disc during regeneration. C: A detail of the dead domain. Note the sharp differences between living tissue (blue network: F-actin-labeled cell contours) and dead tissue (cleaved caspase-3 in red). C': A view of the same disc during regeneration, with cells now covering the dead domain. The genotype of (C) and (C'): *UAS-rpr*; *sal-Gal4*/*tub-Gal80^{ts}*. Scale bar 50 μm.

BioEssays 32:207–217,

reprogramming. In addition, the tools for genetic analysis of *Drosophila* are sophisticated in comparison with those available in other organisms. Moreover, by using different genetic backgrounds, imaginal discs are excellent tissues in which to identify and manipulate early signals that respond to injury and activate regeneration.

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